TABLE 26

Summary of genes Expressed up/down between pregradient and post-gradient (B1-B4) cell populations.

	B1	B2	B3	B4
PreG	165 (32/133) T4/T5	21 (11/10) T6/T7	100 (58/42) T8/T9	227 (201/25) T10/T11
B1		74 (45/28) T12/T13	488 (258/230) T14/T15	534 (359/175) T16/T17
B2			149 (107/42) T18/T19	242 (226/16) T20/T21
B3				30 (27/2) T22/23

[0658] Discussion. The present report describes the genetic profiling of specific bioactive renal cell subpopulations, generated in this case by density gradient separation. Microarray analysis represents an unbiased approach for determining the expression levels of normalized signal intensities in one sample relative to another (data not shown). Cluster analysis and Treeview applications further allow visualization of the intensities and patterns of gene expression across multiple samples (data not shown). The cell fractions examined in the present report have been extensively studied in vivo (Lewis male donors into Lewis female CKD recipients (Kelley et al., 2009)).

TABLE 27

Validation of the Lewis rat microarray results by qrtpcr in the rat and translation of enrichment to gradients of normal human kidney and human CKD kidney Blood Vessel Development

	Sample	Target Gene	Rat RQ	HK19 CKD RQ	HK21 Non-CKD RQ
CDH5	B2	CDH5	0.742	1.052	1.387
	B4	CDH5	8.065	6.205	2.340
KDR	B2	KDR	0.708	0.607	0.233
	B4	KDR	10.348	20.637	6.344
PLAT	B2	PLAT	1.008	1.224	0.430
	B4	PLAT	3.088	4.266	0.430
ANGPT2	B2	ANGPT2	0.737	0.872	0.812
	B4	ANGPT2	6.697	14.115	13.446

[0659] Whole organism improvements (i.e., survival, weight gain), serological profiling and histological evidence overwhelmingly support B2 as the cell fraction with the greatest therapeutic relevance when transplanted alone into diseased kidneys, although fraction B4 did provide limited therapeutic benefits in vivo.

[0660] A follow-up partial factorial in vivo study indicated that combinations of B2 and B4 exceeded the benefits of B2 alone. The present microarray study discovered differences between all cell fractions, with an emphasis on the differences between B2 and B4 that include gene classification through David Annotation freeware (data not shown). Interestingly, genes differentially expressed between B2 and B4 represent 33 different Functional Groups and 163 annotations among these groups with significant p-values ($pv \leqq 0.05$). The array was validated by qrtpcr using a selected panel of genes that represent 7 different David (Go) gene annotation categories (data not shown). Remarkably, the genes selected to validate the rodent array, translated to both a normal human and human CKD patient (see Table 27). As is typical with gene array analyses, caution must be taken in interpretation until all purported markers of the subfractions can be verified at the protein level. Furthermore, rare components of either subfraction are not likely to be detected using gene expression analysis, and must be pursued independently using methods that discriminate on a per-cell basis.

[0661] In conclusion, the microarray analysis validated density gradient separation as an effective means of separating renal cells into subfractions with specific functions and characteristics. Hierarchical Cluster Analysis demonstrated that each subfraction is distinct from all others and from the pre-gradient starting cell population. As shown above, the expression pattern of unfractionated cells is most like that found in the B2 subfraction, likely due to the high frequency (~80%) of tubular and collecting duct cells comprising the heterogeneous culture as well as the B2 subfraction. Also, and as expected, the greatest difference in gene expression occurs between the first and last fractions of the density gradient (B1 vs B4).

[0662] A cluster analysis (based on multiple group comparisons, Kruskal Wallace significance), and a T-test comparison between all groups suggest that fractions B1-B2 separate from B3-B4 in the gradient. The B2 fraction is comprised predominantly of the most plentiful cell(s) in the heterogeneous culture (tubular & collecting duct); only trace quantities of other cell types are present in this fraction, while the B4 fraction is heavily enriched with factors that regulate growth and development, especially blood vessel development. Notably, the B1 and B3 fractions contain immune/inflammatory elements that might offset the therapeutic value of the B2 fraction. The difference in microarray gene expression observed between B2 and B4 was validated by 13/13 rodent markers. Rodent cell fractionation and gene expression strongly translates to both normal and CKD human specimens with >90% of the markers tested. The above data support the proposition that human CKD kidneys are architecturally deficient, but that most cell types are viably present and are propagable ex vivo.

Example 21

Hyaluronic Acid Synthesis by B2

[0663] Surprisingly, HAS-2 (a species of hyaluronic acid synthase responsible for synthesizing high-molecular-weight hyaluronic acid (HA)) was produced by the B2 cell preparation, and to a lesser extent, by the B4 cell preparation in vitro prior to implantation., FIG. **132** shows in vitro expression of HAS-2 by B2 and B4. As shown in FIG. **132**, the predominant expression of hyaluronic acid synthase (HAS) in vitro was in the B2 cell preparation, although there was detectable expression in the B4 cell preparation.

[0664] The in vivo expression of HAS mRNA and protein is shown in FIG. **133**. As shown in FIG. **133**, the implantation of B2 cells into the 5/6 Nx chronic renal failure rodents yielded an upregulation of HAS-2 at the gene level (qRTPCR, bottom